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Toxicity of copper-based antifouling coatings to the marine amphipod, *Eohaustorius estuarius*.

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Atlantic Region
Environment Canada
16th Floor, Queen Square
45 Alderney Drive
Dartmouth, Nova Scotia
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Toxicity of copper-based antifouling coatings to the marine amphipod, *Eohaustorius estuarius.*

By:

W.R. Parker[1], K.G. Doe[2], P. Jackman[2], and J.G. Aubé[2]

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1. Environment Canada, Environmental Protection Branch
Fredericton, New Brunswick
2. Environment Canada, Environmental Conservation Branch
Moncton, New Brunswick

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Abstract

Several environmental surveys in Canada and Europe have determined that the concentration of copper in the sediments around salmon aquaculture sites can be elevated above background concentrations. Copper concentrations in these sediments often exceed the Canadian Council of Ministers of the Environment (CCME) Interim Sediment Quality Guideline (ISQG) for copper (18.7 ug/g on a dry weight basis) and occasionally exceed the Probable Effects Limit (PEL) of 108 ug/g for marine sediments. The most probable source of the copper is the copper-based protective coatings that are applied to the nets used in salmon pen construction. These coatings are intended to eliminate or reduce the fouling of the nets by marine invertebrates and algae. For this study, sediment toxicity tests with the marine amphipod, *Eohaustorius estuaries,* were conducted on sediment samples collected directly under salmon net pens and on sediment samples from a couple of harbours in the local area that had frequent fishing or recreational boat traffic. In addition, samples of clean sediment were spiked with the copper-based anti-fouling coatings Flexgard VI and Flexgard XI to concentrations that were equal to the ISQG, equal to the PEL, and concentrations that exceeded the PEL levels by 5 fold. These spiked sediments were then used to determine the survival of the amphipods over 10 days of exposure. Spiking the reference area sediment with the two Flexgard products at up to five times the PEL of copper did not decrease amphipod survival in the 10-day tests. Copper oxide, the active ingredient in the Flexgard formulations, is insoluble in water and therefore is not likely released from the sediment matrix and not likely bioavailable to the test organisms.

Résumé

Plusieurs études environnementales au Canada et en Europe ont permis de déterminer que la concentration de cuivre autour des sites d'aquaculture de saumon peut être supérieure aux concentrations naturelles. Les teneurs en cuivre de ces sédiments dépassent souvent la valeur spécifiée (18,7 ug/g) dans les recommandations provisoires pour la qualité des sédiments (RPQS) du CCME, et parfois la concentration avec effet probable (CEP), soit 108 ug/g pour les sédiments marins. La source la plus probable de cuivre est constituée par le revêtement protecteur à base de cuivre appliqué sur les filets employés pour l'aménagement des enclos à saumons. Ces revêtements sont conçus pour éliminer ou réduire l'altération des filets par les algues et les invertébrés marins. Dans la présente étude, des essais de toxicité des sédiments pour l'amphipode marin *Eohaustorius estuarius* ont été effectués avec des échantillons de sédiments prélevés directement sous les enclos de filet à saumons ainsi qu'avec des échantillons de sédiments provenant de deux ports de la région connaissant d'importantes activités de pêche et de navigation de loisirs. De plus, des échantillons de sédiments non contaminés ont été enrichis avec des produits de revêtement antisalissures, Flexgard VI et Flexgard XI, à des concentrations respectivement égales à la RPQS et à la CEP, et cinq fois supérieure à la CEP. Ces sédiments ainsi enrichis ont eux aussi soumis à des essais afin de déterminer la survie des amphipodes pendant une exposition de 10 jours. L'enrichissement des sédiments de l'aire de référence avec les deux produits Flexgard à une concentration allant jusqu'à cinq fois la concentration avec effet probable de cuivre n'a pas entraîné de baisse de la survie de l'amphipode au cours de la période expérimentale de 10 jours. Étant donné que l'oxyde de cuivre, qui est la matière active des formulations de Flexgard, est insoluble dans l'eau, elle n'est probablement pas libérée par les sédiments et n'est donc pas biodisponible pour les organismes testés.

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Introduction

Several environmental monitoring surveys of sediments from under marine salmon aquaculture farm sites have identified elevated concentrations of metals, in particular copper and zinc (Burridge et al 1999; Brooks 2001; Parker and Aubé 2002). Sediment concentrations of copper in sediments collected directly beneath salmon net pens regularly exceed interim sediment quality guidelines (ISQG) and occasionally exceed the Probable Effects Level (PEL) established by the Canadian Council of Ministers of the Environment (CCME, 1999). The CCME has set the ISQG for copper at 18.7 ug/g and the PEL at 108 ug/g. Copper was elevated at most marine salmon farm sites surveyed and exceeded the CCME ISQG in 80 % of samples and at 10 of 11 sites sampled (Parker and Aubé 2002). Four of 57 (7 %) samples had copper levels that exceeded the CCME PEL.

Copper is an essential trace element that can be toxic to aquatic organisms at elevated concentrations (Dulka and Risby 1976; Rainbow et al 1990). Because of its affinity for particulate material, copper released to the aquatic environment tends to accumulate in the sediments. Therefore, sediments can act as an important route of copper exposure to marine organisms. Many field studies have been conducted to determine possible environmental impacts from exposure to elevated copper concentrations and the observed adverse biological effects have included decreased benthic community diversity, reduced abundance, increased mortality, biological uptake, lowered reproductive success and behavioral changes (CCME 1999; Ray et al 1981; Deniseger and Kwong 1996; Eriksson and Weeks 1994).

The most likely source of the copper in the sediments is the copper-based antifouling coatings that are used on the salmon farm net pens. These copper-based anti-fouling coatings are applied to the nets to eliminate or reduce the growth of marine invertebrates and algae. The nets are dipped in a copper rich solution before being installed on the farm. Copper does leach from the nets, albeit at a slow rate. Copper that does leach from the net pens would have very low solubility in salt water and would most likely settle out as a precipitate. Copper-based antifouling coatings are also used on commercial fishing boats, aquaculture service boats and recreational boats. The leaching of copper from these vessels could also contribute to the build up of copper in the sediments around salmon farms. The food fed to farm raised salmon contains copper as an essential mineral for the fish. It is also possible that uneaten food and faecal matter, containing copper, may also contribute to the concentrations observed in the sediments around the salmon farms. Brooks (1991) speculated that there was little environmental risk associated with the small amount of copper added to fish food as a micronutrient.

In Canada, two copper-based antifouling products are approved for use on marine aquaculture net pens. These products are manufactured by the Flexabar Corporation of New Jersey, USA and are called Flexgard VI and Flexgard XI. The active ingredient of these two water-based coatings is copper oxide. Flexgard VI contains 15.3% cuprous oxide and Flexgard XI contains 26.5 % cuprous oxide.

This study was conducted to determine if the elevated copper concentrations observed in sediments from under salmon aquaculture sites has the potential to be an environmental hazard to marine organisms.

2 General Study Design

Sediment toxicity studies were conducted to determine if the observed elevated concentrations of copper in sediments under salmon aquaculture net pens could have any adverse effects on marine organisms. Sets of sediment samples were collected from four marine locations in southwest New Brunswick. Since there are other potential sources of copper besides the use on antifouling compounds on aquaculture net pens, the study design called for testing sediments from harbours with extensive boat traffic as well as sediments from an aquaculture site.

These sediment samples were analyzed for copper content, grain size and total organic carbon content prior to undergoing toxicity testing. The toxicity tests were 10-day survival tests using the marine amphipod, *Eohaustorius estuarius*. Past experience with sediments from aquaculture sites (Burridge et al 1999) had indicated that the elevated levels of sulphide and ammonia could cause mortality of the amphipods. In order to remove that artifact from this evaluation, the sediment samples were aerated for 14 days to satisfy the chemical and biological oxygen demand of the organically rich sediments and remove any residual sulphide and ammonia. The four sediment samples were then tested for toxicity with the amphipods.

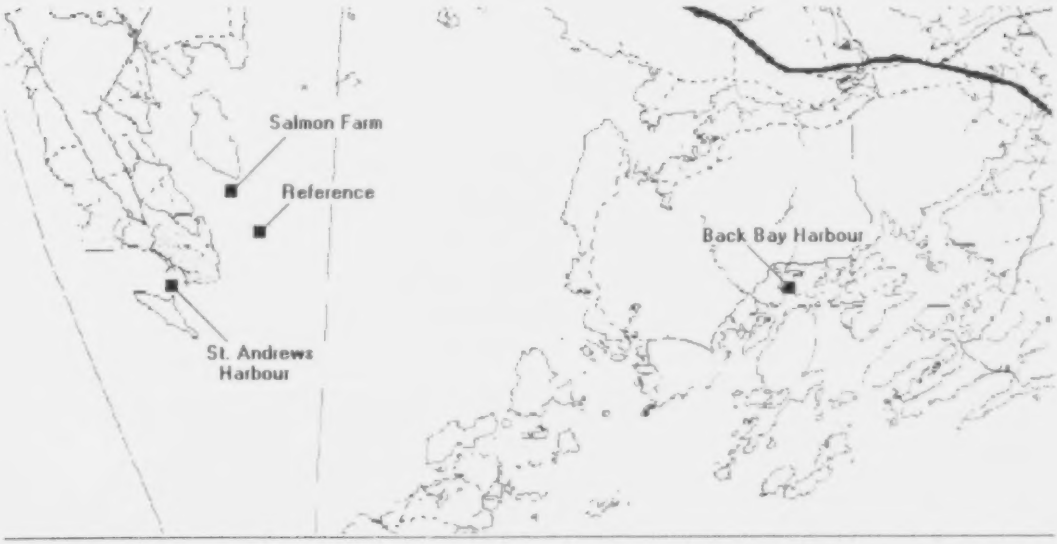
In addition, the reference sediment was spiked with the antifouling coating material to raise the copper concentration to up to 5 times the PEL. Two sets of spiked sediment toxicity tests were conducted, one on each of the antifouling coatings Flexgard VI and Flexgard XI.

3 Detailed Materials and Methods

3.1 Sediment Sampling

On January 9, 2002, sediment samples were collected by SCUBA divers at each of the four sampling locations (Figure 1). The geographical coordinates at each location were determined using a hand held Global Positioning System (GPS) and were recorded. Each sample was scooped from the seafloor using a small hand trowel and placed in 4 litre plastic pail. At the surface, excess water was decanted, the visual characteristics of the sediment were noted and the pail was sealed with a snap on lid. One 4-litre pail of sediment was collected at the salmon farm site, St. Andrews Harbour and Back Bay Harbour. At the reference area, six 4-litre pails of sediment were collected. The pails were labeled and kept cool until transported to the Environment Canada Environmental Quality Laboratory in Moncton.

Figure 1 – Sediment sampling locations in the Bay of Fundy, southwest New Brunswick .



3.2 Sediment Storage and Treatment

Nine sediment samples from four locations were received in the laboratory on 10 January, 2002. Temperature on receipt ranged from 3.5 to 5.0 ^{0}C, and overlying water had a salinity of approximately 32 parts per thousand (ppt).

Samples received were:

1. Block 5, Salmon Farm MF-0290, one 4-L pail (assigned lab number 2002004345);
2. St. Andrews Harbour , one 4-L pail (assigned lab number 2002004347);
3. Back Bay, one 4-L pail (assigned lab number 2002004348);
4. Control, Block 4, six 4-L pails (assigned lab number 2002004346).

The control sediment samples from Block 4, were stored at 4 $^{\circ}$ C until used in the toxicity tests. The other three samples (Block 5, Salmon Farm MF-0290; St. Andrews Harbour; and Back Bay) were treated to remove oxygen demand and sulphides from the sediments. These samples were transferred to 12 L pails on 10 January, through a column of 8 L of vigorously aerating salt water, and continuous vigorous aeration was provided. Sediment settled to the bottom in the pails; the sediment was re-suspended by hand mixing with a stainless steel instrument on Monday, Wednesday, and Friday of each week. This treatment was continued for 14 days. Samples of the sediments for copper analysis were taken after treatment and before the start of the bioassay.

3.3 Sediment Spiking

Three replicates of approximately 6 grams of wet reference sediment were added to tared weighing vessels. The wet weight was recorded, and then the samples were dried at 100°C for 24 hours before determining the dry weight. The average percent moisture was used to determine the quantity of Flexgard required to be added in order to achieve copper concentrations equal to the ISQG, ½ the PEL, the PEL and 5 times the PEL which is reported on a dry weight basis. The required volume of product was added to a weighed quantity of the wet reference sediment in a glass jar and the contents were hand mixed with a stainless steel mixing device. The jars were covered with a Teflon lined lid and placed on an end-over-end mixer for 1 hour at 20 revolutions per minute. After removal from the mixer the sediments were mixed again by hand before transferring to the amphipod test chambers.

3.4 Chemical Analyses

3.4.1 Copper

Sediment samples were homogenized to ensure representative analysis of the individual discrete samples. For the sample analyses, an extraction using microwave assisted processes (MAP) was used (USEPA, 1998). The microwave technology permits the same type of extraction as the "aqua regia" extraction but with faster and more efficient extraction of the metals from the sediments while using lesser volumes of the acids.

Inductively Coupled Plasma - Mass Spectrometer (ICP-MS) is a high capacity, multi-element instrument which requires the addition of internal standards for quantification of the unknowns. First, the samples were diluted 10 fold with double de-ionized water prior to spiking with the internal standards. This was done to reduce the loading of the total dissolved solids onto the detector of the ICP-MS. All the discrete and quality control samples were spiked at the same level with a solution of rare earth elements used as internal standards.

The concentration of copper was determined in each extract and reported on a dry weight basis. A certified reference material (CRM SS-1) was used to establish accuracy and extraction efficiency. The reference material SS-1 is a "Type B naturally contaminated soil (not spiked or fortified) with a particle size of ~ 200 meshes" (SCP Sciences, 1997).

3.4.2 Ammonia, Sulphide and Redox

Sediment samples were thoroughly homogenized and sub-sampled for analysis of sulphide, redox potential (Eh), and ammonia by specific ion electrodes according to the manufacturer's instructions and advice of Dr. B. Hargrave (Dept. of Fisheries and Oceans, Maritimes Region). These analyses were conducted in triplicate and mean and standard deviations were computed. Three sub-samples of the sediment were placed in tared vessels and dried at 100°C for 24 hours; these weights were used to convert results to a dry weight basis. Results for sediments are expressed as µg S/g dry weight of sediment for sulphide, µg NH_3-N/g dry weight of sediment for ammonia and millivolts corrected for the normal hydrogen electrode for redox potential.

In addition to the analysis of the sediment samples, the level of ammonia was measured in the overlying water in the amphipod assay at the start and end of the test. The amount of unionized ammonia, based on the test temperature, salinity and pH, was calculated according to Bower and Bidwell (1978).

3.5 *Eohaustorius estuarius* Sediment Toxicity Test Protocol

Eohaustorius estuarius are amphipods that are used to test sediment samples that were essentially estuarine or marine (salinity greater than 2 parts per thousand or ppt). This species can be used to test sediments with up to 70% clay and 100% fine particles (Environment Canada, 1998). This amphipod sediment toxicity test is commonly used for assessment of sediment contamination in Canada and the United States (Environment Canada 1992 and Environment Canada 1996).

Eohaustorius estuarius were collected 26 January 2002 by NAS Associates at Beaver Creek, Oregon, USA, and were received at EQL, Moncton on 30 January, 2002. The conditions upon receipt were: 9.2 to 9.8 °C and 25-26 ppt salinity. The animals were acclimated to and maintained at 15 ± 2°C and 30± 2 ppt salinity until used for testing of the sediments on February 1 and February 5, 2002.

Methods used followed Environment Canada 1992. On the day prior to starting the test, a 4L bucket of each test sediment was homogenized and 175 mL portions were added to each of five 1L glass mason jars. The jars were filled with 800 mL of clean water of 28 ppt salinity, covered, then aerated overnight with oil free compressed air at a rate of approximately 150 mL /minute.

The overlying water from two replicates of each test sediment was tested for temperature, dissolved oxygen, salinity and pH. A sample of the overlying water was taken from each replicate and combined. This combined solution was tested for ammonia and un-ionized ammonia was calculated based on pH, salinity and temperature (Bower and Bidwell 1978).

The amphipods were sieved from their holding sediment. Twenty animals were added to each of the test vessels. Aeration was ceased for 30 minutes to facilitate burrowing. Testing was performed with a 24 hour light photoperiod with lighting provided by overhead fluorescent fixtures. Testing was performed at 15 ± 1°C. Tests were checked daily for mortalities, evidence of burrowing, aeration and temperature. Three times a week a replicate of each sample was monitored for pH, salinity and dissolved oxygen. After 10 days, the contents of each jar were sieved through a 0.5 mm sieve. Any immobile animals were observed under a microscope to determine mortality, which was defined as lack of all movement when observed under a dissecting microscope for 5 - 10 seconds. Any animals missing were assumed to be dead. The mean survival and standard deviation of each treatment was calculated.

Mean percentage survival of amphipods exposed to the test sediment was compared to mean percent survival of amphipods exposed to the clean field reference sediment, or when this was not tested, to collection site control sediment. If survival in the test sediments was more than 20 % lower than mean survival in the reference site sediment, or 30 % lower than mean survival in the collection site control sediment, and statistically different, then the sample was considered toxic (Environment Canada 1996 and Environment Canada 1998).

A reference toxicant test was conducted with cadmium chloride using water only exposures for 96-hours duration in constant dark. Testing was started on February 1, 2002. Using the mortality data at each test concentration, the 96-hour LC50 (concentration calculated to cause 50% mortality after 96-h exposure) was calculated using the methods of Stephan (1977). The value was entered into the control chart to determine if normal response patterns had occurred and that the population of amphipods used in the test was of normal sensitivity.

4 Results

4.1 Sediment Sampling

The geographical coordinates for the four sample locations are provided in Table 1 along with a description of the physical appearance of the sediments. One sample was collected from directly under a salmon aquaculture farm site in Passamaquoddy Bay (Figure 1). This farm is located in a depositional area and has a history of having organic material building up under the net pens. The material sampled was fine grained and black in colour. The second sample was collected from St. Andrews Harbour, a busy harbour with daily boat traffic using the large wharf and a mooring field for recreational boats, fishing vessels, aquaculture service vessels and whale watching tour boats. The sediment sampled from this location was light brown in colour and consisted of a sand and silt mixture. The third sample was collected from Back Bay Harbour, an active fishing and aquaculture service harbour located to the east of Passamaquoddy Bay. The material collected was fine grained and black in colour. The final sample was collected from a reference area in Passamaquoddy Bay located several hundred metres from any salmon farm. Samples previously checked at this location (Parker and Aubé 2002) had background concentrations of copper. The material collected was dark brown in colour and consisted of fine grain mud.

4.2 Chemical Analyses

The sediment samples were measured to determine total organic carbon (TOC) content, grain size and copper concentration. The TOC results are presented in Figure 2 and indicate that the St. Andrews sediment had the lowest level of TOC at 1.0 %. The sediment from the reference area had 1.8 % TOC and the salmon farm sediment and Back Bay sediment were quite similar with 2.2 % and 2.7 % TOC respectively.

The grain size results are provided in Figure 3. The results indicate that the sediments from Back Bay and from the salmon farm were similar and consisted primarily of a silt fraction (about 70 %) with about 20 % clay. The reference area sediment was similar to these but with a slightly higher clay fraction (about 30 %). The St. Andrews Harbour sediment was different from the others and was primarily sand (70 %) with about 15 % each of silt and clay.

The results of the analyses for copper in the sediments are provided in Table 2. St. Andrews Harbour sediment had lowest copper concentration at 8.0 ug/g. The reference area and the salmon farm site had very similar copper concentrations near 20 ug/g. Back Bay sediment had an elevated copper concentration of 36.7 ug/g, nearly double the ISQG established by the CCME.

Table 1 – Location and description of sediment samples collected in the Bay of Fundy, southwest New Brunswick on January 9, 2002.

Sample Location	Latitude	Longitude	Appearance
Salmon Farm	45° 05.614 N	067° 02.153 W	Fine grain silt, black in colour
Reference Area	45° 05.610 N	067° 02.310 W	Fine grain mud, dark brown in colour
St. Andrew's Harbour	45° 04.139 N	067° 03.315 W	Sandy/silt mixture, light brown in colour
Back Bay Harbour	45° 03.370 N	066° 51.790 W	Fine black mud

The objective for the spiked sediment tests was to have a gradient of five copper concentrations from background levels up to five times the PEL. A target copper concentration gradient equal to the control sediment (20 ug/g), the ISQG concentration (18.7 ug/g), one-half of the PEL concentration (54 ug/g), the PEL concentration (108 ug/g) and five times the PEL concentration (540 ug/g) was selected. The results of the copper analyses on the sediment samples that were spiked with the two antifouling coatings are provided in Table 3 and Figure 4.

As indicated by the results, a good gradient of sediment copper concentrations was achieved for the toxicity exposures although the precision at achieving the target concentration was quite variable.

The results of the ammonia, sulphide and redox analysis are provided n Table 4. For the three sediments that were treated to reduce the ammonia and sulphide levels, measurements were made prior to treatment and after treatment. The salmon farm site did not show a decrease in ammonia with the treatment and Back Bay samples had significant decreases in both ammonia and sulphide with the treatment (Table 5). At the start and end of the amphipod test, a sample of the overlying water was taken and the ammonia level was measured. The levels of ammonia in the overlying water were low and not at a level of concern for this species.

Figure 2 – Total organic carbon content of sediment samples collected in the Bay of Fundy in January 2002.

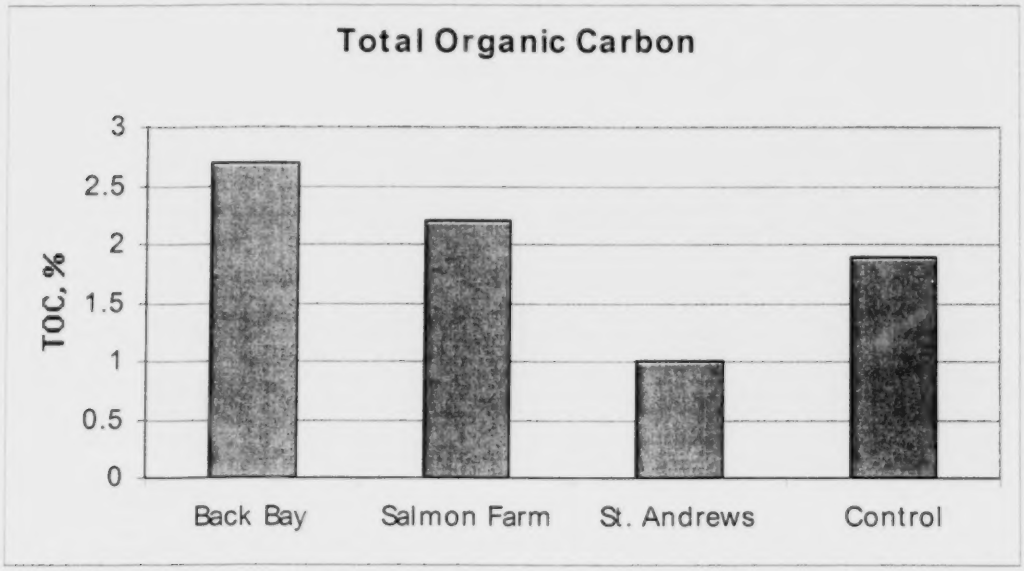


Figure 3 – Grain size analyses of sediment samples collected in the Bay of Fundy in January 2002.

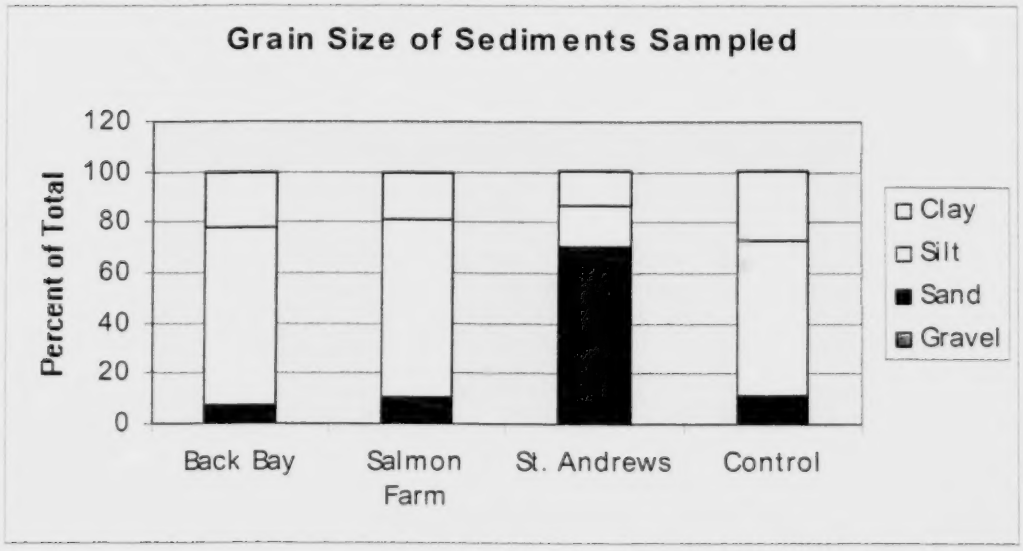


Table 2 – Total copper concentration in sediment samples collected from the Bay of Fundy in January 2002.

Sample Location	Total Copper (ug/g)
Salmon Farm	21.1
St. Andrews Harbour	8.0
Back Bay Harbour	36.7
Reference Area	19.9

Table 3 – Total copper concentrations and the percent of target total copper concentration achieved by spiking reference area sediments with Flexgard VI and Flexgard XI, two copper-based antifouling coatings.

Exposure for Amphipod Survival Tests	Target Concentration of copper, ug/g	Flexgard VI – Actual copper concentration, ug/g (percent actual to target concentration in brackets)	Flexgard XI – Actual copper concentration, ug/g (percent actual to target concentration in brackets)
Control	20	19.9 (99.5)	19.9 (99.5)
ISQG	18	32.9 (182.8)	36.0 (200.0)
Half of the PEL (0.5 X PEL)	54	69.5 (128.7)	44.2 (81.9)
PEL	108	100.9 (93.4)	61.0 (56.5)
Five times the PEL (5 X PEL)	540	497.7 (92.3)	323.8 (60.0)

Table 4 - Ammonia, Sulphide and Redox in Sediments (Mean and Standard Deviation)

Sample	Ammonia µg/g NH_3-N	Sulphide µg/g S	Redox mV
Amphipod Control Sediment	0.2 ± 0.1	0.1 ± 0.1	213 ± < 1
Reference sediment	12.8 ± 0.2	69.9 ± 6.5	-86 ± < 1
Salmon Farm Before treatment	51.9 ± 0.5	95.7 ± 8.0	-76 ± < 1
Salmon Farm After treatment	48.3 ± 1.3	140 ± 5	-92 ± < 1
St. Andrew's Harbour Before treatment	15.3 ± 1.3	23.3 ± 1.0	-94 ± < 1
St. Andrew's Harbour After treatment	2.2 ± 0.2	7.0 ± 0.8	-60 ± < 1
Back Bay Harbour Before treatment	13.6 ± 0.4	79.8 ± 5.0	-125 ± < 1
Back Bay Harbour After treatment	9.5 ± 0.2	6.5 ± 0.5	-94 ± < 1

Table 5 - Ammonia in overlying water and beginning and end of amphipod test (mg/L NH_3-N)

Sample	T=0 days		T=10 days	
	Total	Un-ionized	Total	Un-ionized
Amphipod Control	0.39	0.01	0.35	0.01
Reference Site	0.47	0.01	0.30	< 0.01
Salmon farm	0.81	0.01	2.94	0.03
St. Andrew's Harbour	0.32	0.01	0.25	< 0.01
Back Bay	0.35	0.01	0.32	< 0.04

Figure 4 – Target and measured copper concentrations in sediment spiked with Flexgard VI, a copper-based antifouling coating.

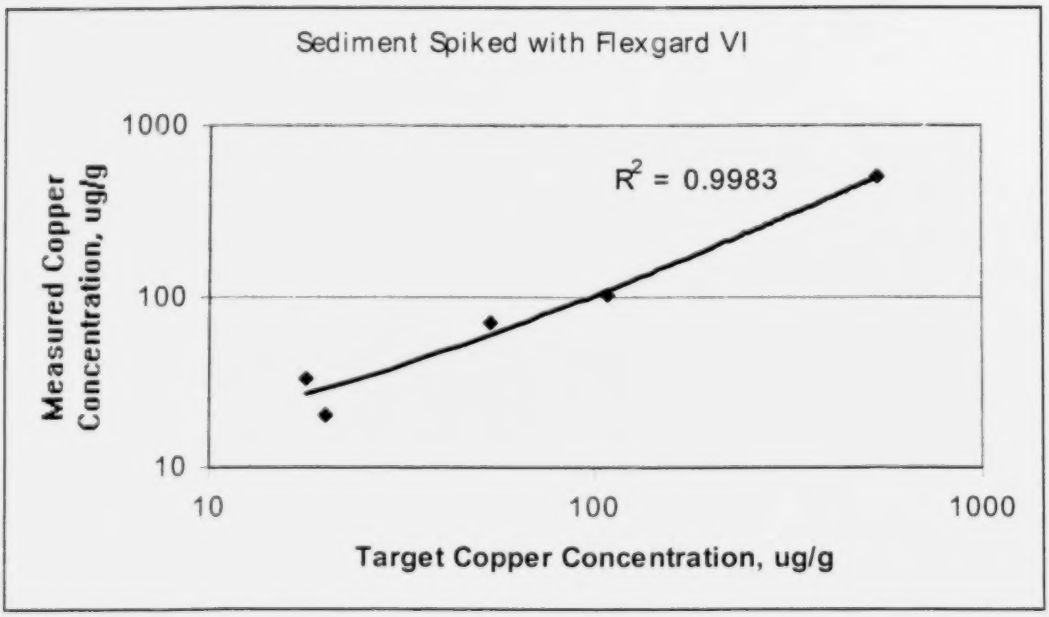
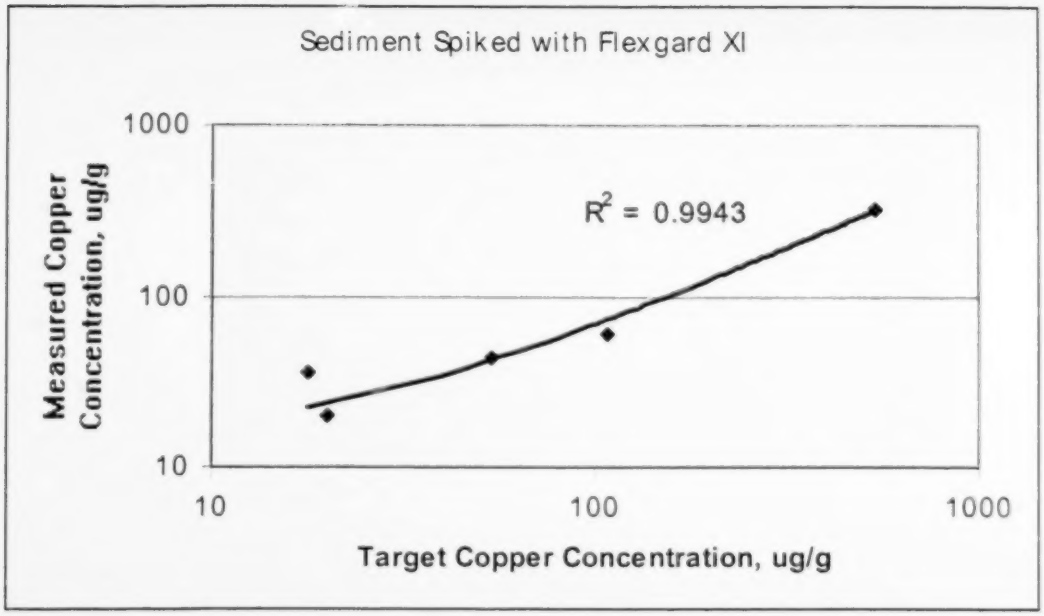


Figure 5 – Target and measured copper concentrations in sediment spiked with Flexgard XI, a copper-based antifouling coating.



4.3 Amphipod Survival Tests

Results of tests conducted on the four sediment samples collected on January 9, 2002 after the samples, following aeration to reduce sulphide and ammonia, are summarized in Figure 6 and the full data are provided in the appendix. The control sediment had 100 % survival of the amphipods in all 5 replicates. The sediments from Back Bay, the salmon farm and St. Andrews Harbour all had mean survival of around 85 % while the reference area sediment had a mean survival of 64 %. There was no significant difference between the survival among the 5 sediments ($p = 0.05$).

The results of the amphipod survival tests on the reference sediment spiked with Flexgard VI are summarized in Figure 7 and the full data are provided in the appendix. The mean survival of the amphipods to the sediments ranged from 64 % to 79 % and there was no significant difference in survival among the five exposures.

The results of the amphipod survival tests on the reference sediment spiked with Flexgard XI are summarized in Figure 8 and the full data are provided in the appendix. The mean survival of the amphipods to the sediments ranged from 54 % to 83 %. There was no significant difference in survival among the five exposures.

Figure 6 – 10-day survival of the amphipod, *Eohaustorius estuarius*, in sediment samples from the Bay of Fundy.

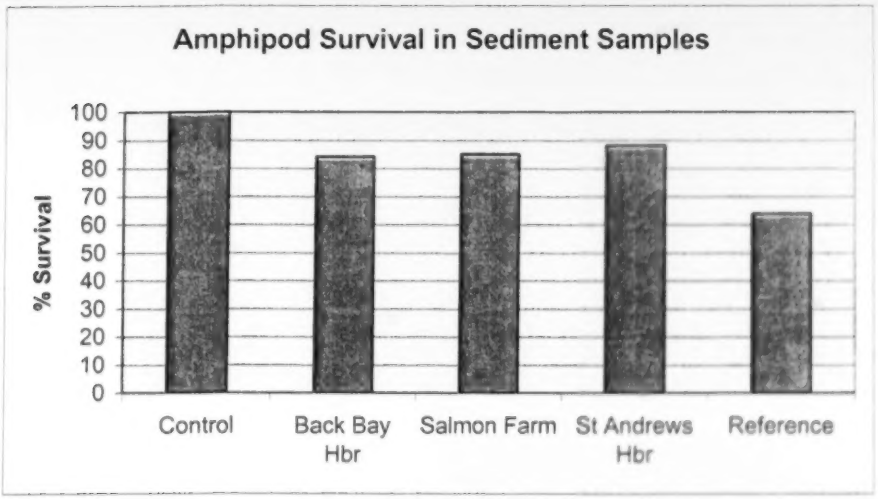


Figure 7 - Survival (Mean and standard deviation) of the amphipod, *Eohaustorius estuarius*, after 10-days exposure to sediment samples from the Bay of Fundy which were spiked with the copper-based antifouling coating, Flexgard VI.

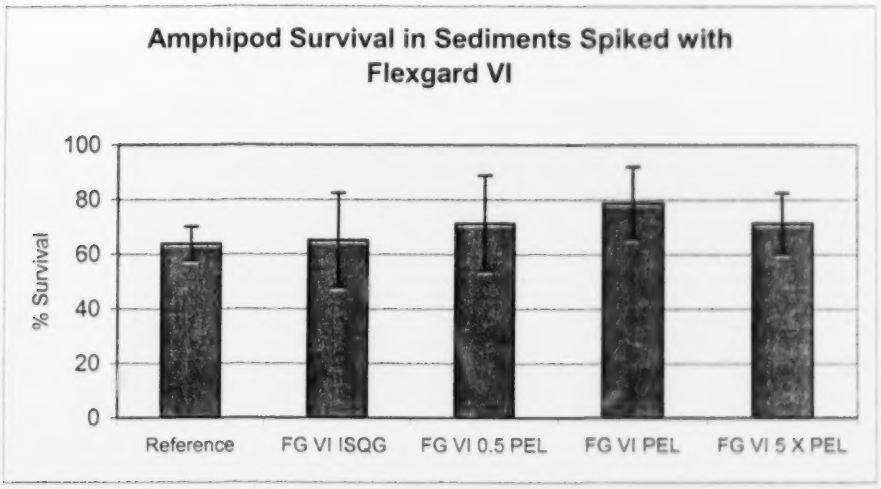
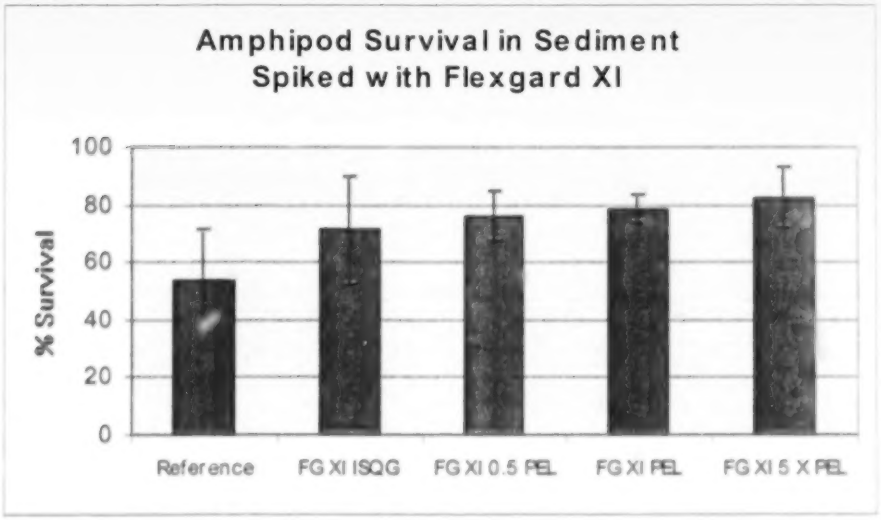


Figure 8 - Survival (Mean and standard deviation) of the amphipod, *Eohaustorius estuarius*, after 10-days exposure in sediment samples from the Bay of Fundy which were spiked with copper-based antifouling coating, Flexgard VI.



5.0 Discussion

5.1 Bay of Fundy Sediments

Previous studies have identified a consistent pattern of elevated copper in sediments under marine salmon aquaculture sites (Burridge, et al 1999; Parker and Aubé, 2002 ; Brooks, 2001). The inference has been made that this elevated sediment copper is related to the use of copper-based antifouling coatings on the netting of the salmon net pens. There are other potential sources of copper to the marine environment around salmon aquaculture areas. These include dietary copper added to salmon food and the use of copper as antifouling coatings on marine vessels. This study also looked at sediments from two areas with active boat traffic (Back Bay and St. Andrews Harbour). The sediment collected from Back Bay had elevated copper and that sampling location was more than 500 metres from the nearest salmon farm. Therefore, it is apparent that copper in marine sediments can originate from other sources besides the antifouling coating on salmon net pens.

Three of the four samples collected were quite similar in colour, grain size and total organic carbon content. The sediment in St. Andrews Harbour was courser and had lower TOC and copper. The courser sediment generally would indicate an erosional environment so even with the large amount of boat traffic and the presence of approximately 50 boats in the mooring field, copper was very low in the sediment. This may indicate that the observed elevation of copper concentrations in marine sediments is related to the depositional nature of the areas as indicated by the presence of fine grain sediments and higher organic carbon content.

The sediments from the salmon farm, St. Andrews Harbour and Back Bay Harbour were all aerated for 14 days prior to the start of the amphipod test to reduce the sediment oxygen demand

and remove hydrogen sulphide and ammonia from the test solutions. The sediment from the reference area was not pre-treated in this manner. None of the four sediment samples caused a significant reduction in the 10-day amphipod survival compared to the standard control sediment from Oregon, USA where the test amphipods are collected. The amphipod survival in the reference area sediment was the lowest ranging from 52 % to 62 % but this was not a significant reduction from the control. The reference sediment was in many respects quite similar in colour, grain size, ammonia, sulphide, and total organic carbon content to the other three samples collected. This suggests that it was not collected from a pristine site, and likely received some inputs from the same sources as the salmon farm sediments. Since it was not pre-treated by aeration for 14 days prior to the start of the amphipod test to reduce ammonia and sulphide, this could account for the lower survival observed.

5.2 Flexgard Spiked Sediments

The sediment collected from the reference area was used as the base sediment for the preparation of the spiked sediments. This sediment consisted primarily of silt (60 %) and clay (30 %). The sediment had about 2 % total organic carbon and 19.9 ug/g of total copper. The Flexgard VI contains 15.3 % copper oxide and the Flexgard XI consists of 26.5 % copper oxide. By adding the Flexgard materials to the sediment and thoroughly homogenizing the mixture; a series of sediments with a gradient of copper concentrations was achieved (Figure 4 and Figure 5). The highest copper concentration for the Flexgard VI spiked sediment was 323.8 ug/g, which was 17.3 times the ISQG and 3.0 times the PEL. For the Flexgard XI spiked sediments, the highest concentration had 497.7 ug/g of copper, which was 26.6 times the ISQG and 4.6 times the PEL. Neither antifouling product caused a reduction in the 10-day survival rate of the amphipods even at the highest copper concentrations when compared to the unspiked reference sediment.

With copper concentrations that were 3 to 5 times the PEL, a toxic response may well have been expected. There may be several reasons for the lack of toxicity. The active ingredient in the Flexgard formulations is copper oxide, which is insoluble in water. Copper must be in a dissolved form to reach and pass through biological membranes and act on organs and tissues in an aquatic organism. Insoluble forms of copper cannot transfer through these membranes so as long as the copper remains insoluble; it should not cause a toxic reaction (Ray et al 1981). Copper in the aquatic environment is known to have an affinity for fine grained organic material (Loring 1981) and the reference area sediment used for these experiments was about 90 % fine-grained material with a 2 % TOC content. So any copper that became dissociated from the netting and was deposited on the bottom would likely form a strong affinity for the sediments and remain there in an insoluble form.

Finally, copper is primarily considered an acute toxicant. Copper attacks gill membranes in aquatic organisms and interferes with the transport of oxygen across those gills causing hypoxia and death. Several studies have reported stimulatory effects on growth and reproduction of low level copper exposures (called "hormesis") (Stebbing 1981 and 1982). Many animals can metabolize and depurate copper and therefore copper does not bioaccumulate but is regulated in animal tissues (Rainbow and White 1989; McKim and Benoit 1974). There is evidence of chronic and sublethal toxicity in aquatic animals caused by copper (McKim and Benoit 1971) and the 10-day amphipod survival test used in this study would not assess those types of responses. Further evaluation of the potential for the elevated copper to have negative chronic or

sublethal effects on marine biota should be conducted with tests appropriate for those types of assessment. The results of the current study clearly indicate that there was not any acute mortality of the amphipods even at copper concentrations more than an order of magnitude over those levels observed in salmon farm sediments.

6.0 Conclusions

1) Spiking the reference area sediment with the two Flexgard products (VI and XI) at up to 5 times the PEL as Cu did not decrease amphipod survival in the 10-day tests.

2) Copper oxide, the active ingredient in the Flexgard formulations, is insoluble in water and therefore is not likely bioavailable.

3) The elevated levels of copper frequently measured in sediments collected near salmon aquaculture sites was not acutely lethal to marine amphipods.

7.0 Recommendations

1) Although these tests indicated that there was no acute lethality to the amphipods, sublethal testing and chronic testing of copper-based antifouling coatings should be conducted to help assess the risk of copper to marine species.

2) Studies should be conducted to examine the pathways for the transport of copper from salmon net pens to marine sediments.

3) Continued monitoring of copper concentrations in sediments under and around marine aquaculture sites should be conducted to follow trends.

4) Environment Canada should promote research and development into alternative techniques means for minimizing the biofouling of salmon aquaculture net pens.

8.0 Acknowledgements

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Appendix

Appendix Table 1 – Percent survival of the marine amphipod, E. estuarius, following 10-days exposure to marine sediment samples from the Bay of Fundy.

Test Exposure	Total Copper, ug/g	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD
Laboratory Control	n/a	100	100	100	100	100	100	0.00
Back Bay Harbour	36.7	90	85	70	85	90	84	8.22
Salmon Farm	21.1	95	80	85	80	85	85	6.12
St. Andrew's Harbour	8	85	85	100	85	85	88	6.71
Reference Area	19.9	55	70	65	65		64	6.3

Appendix Table 2 – Percent survival of the marine amphipod, E. estuarius, following 10-days exposure to reference area sediment spiked with Flexgard VI.

Test Exposure	Actual Copper, ug/g	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Flexgard VI Control	19.9	55	70	65	65	63.75	6.29
Flexgard VI 18.7	32.9	80	50	80	50	65	17.32
Flexgard VI 52	69.5	80	80	45	80	71.25	17.50
Flexgard VI 108	100.9	85	90	60	80	78.75	13.15
Flexgard VI 540	497.7	75	55	75	80	71.25	11.09

Appendix Table 3 – Percent survival of the marine amphipod, E. estuarius, following 10-days exposure to reference area sediment spiked with Flexgard XI.

Test Exposure	Actual Copper, ug/g	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Laboratory Control	n/a	100	90	100	95	96.25	4.79
Flexgard XI Control	19.9	45	45	80	45	53.75	17.50
Flexgard XI 18.7	36	55	85	55	90	71.25	18.87
Flexgard XI 52	44.2	85	75	80	65	76.25	8.54
Flexgard XI 108	61	75	80	85	75	78.75	4.79
Flexgard XI 540	323.8	70	85	80	95	82.5	10.41